REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

The objection to claim 19 is respectfully traversed in view of the above amendments.

The objection to claims 6, 8, 10, 15, 57, 58, and 62 as being dependent on rejected base claims is respectfully traversed. Applicant submits that there is no need to rewrite these claims in independent form, because, for the reasons noted *infra*, the claims from which they depend are patentable.

The rejection of claims 17, 55, and 60 under 35 U.S.C. § 112, second paragraph, for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 5, 7, 12, 13, 14, 16, 19, 20, 54, 59, 64, and 65 under 35 U.S.C. § 112, first paragraph, for lack of written description is respectfully traversed. Pursuant to the Guidelines for Examination of Patent Applications Under the 35 U.S.C. § 112, ¶1, "Written Description" Requirement, 66 Fed. Reg. 1099-1111, 1106 (January 5, 2001), the written description requirement may be satisfied through sufficient description of a representative number of species by a combination of identifying characteristics which are sufficient to show the applicant was in possession of the claimed genus. Whether the described species constitute a representative number of species is determined by assessing whether the described species are representative of the entire genus (Id.).

Applicant submits that: (1) the presently claimed genus of a DNA molecule encoding the protein δ' subunit of polymerase III holoenzyme comprising a nucleic acid sequence which hybridizes to a nucleotide sequence corresponding to SEQ. ID. No. 11 or SEQ. ID. No. 12 or SEQ. ID. No. 13 when hybridization is performed in 2 x SSC, 0.2% SDS at 42 °C and the resulting protein subunit, and (2) the presently claimed genus of a DNA molecule encoding the protein δ subunit of polymerase III holoenzyme comprising a nucleic acid sequence which hybridizes to a nucleotide sequence corresponding to SEQ. ID. No. 6 when hybridization is performed in 2 x SSC, 0.2% SDS at 42 °C and the resulting protein subunit, are adequately represented by the species which are taught and described in the present application (i.e., the DNA molecules encoding the δ' and δ subunits for *E. coli* and the subunits themselves), because the genus's do not embrace widely variant species and the

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disclosed species exhibit the shared structural and functional characteristics of the claimed genus's.

It is well known to one skilled in the art that proteins homologous to the δ and δ' subunits of the *E. coli* polymerase III holoenzyme are contained in organisms other than *E. coli*, as shown in the Declaration of Michael O'Donnell under 37 CFR § 1.132 submitted in parent U.S. Patent Application Serial No. 08/279,058 on December 17, 1996 ("Supp. O'Donnell Declaration") and the Supplemental Declaration of Michael O'Donnell under 37 CFR § 1.132 ("Supp. O'Donnell Declaration") previously submitted in the present application on June 8, 1999.

Those skilled in the art recognize the δ' subunit from *E. coli* has sequence homology to accessory protein complexes of various other organisms (O'Donnell Declaration ¶ 13). For example, in O'Donnell et al., "Homology in Accessory Proteins of Replicative Polymerases - *E. coli* to Humans," Nucleic Acids Research 21(1):1-3 (1993), a comparison of amino acid sequences shows the homology between proteins of replicative polymerases of *E. coli*, humans, and phage T4 (Id.). In Carter et al., "Identification, Isolation, and Characterization of the Structural Gene Encoding the δ' Subunit of *Escherichia coli* DNA Polymerase III Holoenzyme," J. of Bacteriology, 175(12):3812-22 (1993), Figure 5 diagrams the homology of the δ' amino acid sequence to other replication proteins (Id.). Comparison of the δ' amino acid sequence revealed similarity to the A1(replication factor C) complex of HeLa cells and to the gene 44 protein (gp44) of bacteriophage T4 (Id.). In addition, amino acid sequence similarity was found to the gene product of *B. subtilis* (Id.). Further, the structural homology of the δ' subunit to other replication proteins has been proven to be true (Id.). For example, the genome project of *Haemophilus influenzae* showed homologues to all 10 subunits of *E. coli* DNA polymerase III holoenzyme, including δ and δ' (Id.). Currently, the GenBank now also shows homologues to the δ' subunit of *E. coli* from a large variety of organisms, including the following prokaryotes: *Escherichia coli, Haemophilus influenzae, Micrococcus luteus, Pseudomonas aeruginosa, Bacillus subtilis,* and *Caulobacter crescentus* (Id.).

As to the δ and δ' subunits of polymerase III holoenzyme, it is well known to one skilled in the art that proteins in other organisms have functional and structural homology to the subunits of *E. coli* (O'Donnell Declaration ¶¶ 10-16).

Various genome projects for many different organisms have resulted in the gene sequences for various bacteria being publicly available on various web sites (Supp.

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O'Donnell Decl. ¶5). As described more fully below, the amino acid sequences for the δ and δ' subunits for *E. coli*, disclosed in the present application, were used, by myself and others, in a BLAST search program (Altschul, et al., "Basic Local Alignment Search Tool," J. Mol. Bio. 215:403-10 (1990)) to identify the presence of genes encoding these proteins in other eubacterial prokaryotes (Id.). The results of these analyses are set forth below (Id.).

The sequence analysis of *Haemophilus influenze* is found at http://www.tigr.org/tdb/mdb/hidb/hidb.htm1 (Supp. O'Donnell Decl. ¶6). A copy of that web site listing is attached to the Supp. O'Donnell Decl. at Appendix B with the δ subunit encoding gene being identified as HI0923 and the δ' subunit encoding DNA molecule being identified as HI0455 (Id.). This listing shows that the δ subunit encoding DNA molecule of *Haemophilus influenze* is 62.0% similar to the δ subunit encoding DNA molecule of *E. coli* (Id.). Likewise, the δ' subunit of *Haemophilus influenze* is shown to be 57.4% similar to the δ' subunit encoding DNA molecule of *E. coli* (Id.).

The genome of *Niceria gonorrhoeae* is found at the web site http://www.genome.on.edu (Supp. O'Donnell Decl. ¶7). Search for the δ subunit amino acid sequence yields a contig. with a very high probability of 1.2×10^{-25}, contig. 188, while the δ' amino acid sequence yields a contig. of high probability of 1.2×10^{-14} #200 (Id.). See Appendix C attached to the Supp. O'Donnell Decl.

The genome for *Shewanella putrefaciens* is found on the TIGR BLAST server (Supp. O'Donnell Decl. ¶8). A search for the δ subunit produced the high score of 1.1×10^{-54} for contig. gsp 230, while the search for δ' subunit produced the high score of 6.4×10^{-27} for contig. gsp 271 (Id.). See Appendix D attached to the Supp. O'Donnell Decl.

The genome for *Vibrio cholerae* is found at http://www.tigr.org/cgi-bin/BlastSearch/blast.cgi?organism=v.cholerae (Supp. O'Donnell Decl. ¶9). A search for the δ subunit produced the high score of 6.9×10^{-82} for contig. asm 937, while the search for δ' subunit produced the high score of 8.1×10^{-37} for contig. asm 894 (Id.). See Appendix E attached to the Supp. O'Donnell Decl.

The genomes for *Pseudomonas aeruginosa* (see Appendix F attached to the Supp. O'Donnell Decl.), *Salmonella typhi* (see Appendix G attached to the Supp. O'Donnell Decl.), and *Yersinia pestis* (see Appendix H attached to the Supp. O'Donnell Decl.) are found at http://www.ncbi.nlm.nih.gov/Blast/unfinished genomes (Supp. O'Donnell Decl. ¶10). For these, the amino acid sequences of *E. coli* δ and δ' were used in BLAST searches (Id.). The

high scores, given below, are all sufficiently significant to call the identified gene the one that performs the homologous function in *E. coli* (Id.):

Pseudomonas aeruginosa
δ - 7×10^{-34} contig. 52
δ' - 9×10^{-27} contig. 50

Salmonella typhi
δ - 1×10^{-161} contig. 1564
δ' - 8×10^{-10} contig. 870

Yersinia pestis
δ - 1×10^{-127} contig. 803
δ' - 9×10^{-98} contig. 51

Thus, for Gram negative bacteria such as *Haemophilus influenze*, *Niceria gonorrhoeae*, *Shewanella putrefaciens*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Yersinia pestis*, there is a high level of homology between the δ and δ' subunits of those bacteria and the δ and δ' subunits of *E. coli* (Supp. O'Donnell Decl. ¶11).

For other eubacteria, there is significant homology between their δ' subunit and that of *E. coli* (Supp. O'Donnell Decl. ¶12). In all eubacteria, the δ subunit can be identified starting with the *E. coli* δ subunit as comparison, but, since it is not as conserved as the δ' subunit, one must "walk" from one organism to another, as discussed in ¶ 23 of the Supp. O'Donnell Decl. (Id.).

In Himmelreich et al., "Complete Sequence Analysis of the Genome of the Bacterium *Mycoplasma pneumoniae*," Nucleic Acids Research 24(22):4420-4449 (1996), the δ' subunit of *Mycoplasma pneumoniae* is identified as being homologous to the δ' subunit of *E. coli* in Table 1 on page 4426 (Supp. O'Donnell Decl. ¶13). See Appendix I attached to the Supp. O'Donnell Decl.

In Kunst et al., "The Complete Genome Sequence of the Gram-positive Bacterium *Bacillus subtilis*," Nature 390:249-256 (1997), the δ' subunit of *Bacillus subtilis* is identified as being homologous to the δ' subunit of *E. coli* in the table on page 248 (Supp. O'Donnell Decl. ¶14). See Appendix J attached to the Supp. O'Donnell Decl.

The genome for *Streptococcus pyogenes* is found in the University of Oklahoma server (i.e. http://www.ncbi.nlm.nih.gov.BLAST/tigrbl.html) (Supp. O'Donnell

Decl. ¶15). δ' produced the high score of 3.3×10^{-10} for contig. 218 (Id.). See Appendix K attached to the Supp. O'Donnell Decl.

The genome for *Enterococcus faecalis* is found on the TIGR BLAST search server (Supp. O'Donnell Decl. ¶16). δ' produced the high score of 9.6×10^{-16} for contig. 6277 (Id.). See Appendix L attached to the Supp. O'Donnell Decl.

The genome for *Streptococcus pneumoniae* is found on the TIGR BLAST search server (Supp. O'Donnell Decl. ¶17). δ' produced the high score of 2.4×10^{-12} for contig. sp 68 (Id.). See Appendix M attached to the Supp. O'Donnell Decl.

The genome for *Aquifex aeolicus* is found in Deckert et al., "The Complete Genome of the Hyperthermophilic bacterium *Aquifex aeolicus*," Nature 392:353-358 (1998) and at http://www.ncbi.nlm.nih.gov/Blast/unfinished genomes (Supp. O'Donnell Decl. ¶18). δ' produced the high score of 8×10^{-13} (position 1303996-1304394) (Id.). See Appendix N attached to the Supp. O'Donnell Decl.

The genome for *Thermatoga maritima* is found in the TIGR BLAST server page (Supp. O'Donnell Decl. ¶19). δ' yields a high score of 3.7×10^{-15} for contig. tm 26 (Id.). See Appendix O attached to the Supp. O'Donnell Decl.

In *Spirochaetes*, Tomb et al., "The Complete Genome Sequence of the Gastric Pathogen *Helicobacter pylori*," Nature 388:539-547 (1997) (see Appendix P attached to the Supp. O'Donnell Decl.) and Fraser et al., "Genomic Sequence of a Lyme Disease Spirochaete, *Borrelia burgdorferi*," Nature 390:580-586 (1997) (see Appendix Q attached to the Supp. O'Donnell Decl.), *Helicobacter pylori* and *Borrelia burgdorferi* are identified to have δ' subunits (Supp. O'Donnell Decl. ¶20). For *Helicobacter pylori*, δ' is listed in the table as HP1231 (Id.). For *Borrelia burgdorferi*, using the NCBI genome search page (Ncbi.nlm.nih.gov/Blast/unfinished genomes), δ' gives the high score of 8×10^{-7} (Id.). See Appendix R attached to the Supp. O'Donnell Decl.

In Andersson et al., "The Genome Sequence of *Rickettsia prowazekii* and the Origin of Mitochondria," Nature 396:133-140 (1998), *Rickettsia prowazekii* is identified to have a δ' subunit, identified as RP172 (Supp. O'Donnell Decl. ¶21). See Appendix S attached to the Supp. O'Donnell Decl.

A large compilation of genome sequences is at the web site http://www.ncbi.nlm.gov/Blast/unfinished genome.html (Supp. O'Donnell Decl. ¶22). The eubacterial genomes were searched using the δ' subunit of *E. coli* (Id.). All organisms in eubacteria scored very high with identity levels sufficient to identify the holB gene encoding

δ' conclusively (Id.). This is seen in Figure 1 showing a path of one-on-one comparative alignments each of which start with *E. coli* and the alignments (Id.) (Appendix T attached to the Supp. O'Donnell Decl.). In this figure, within the parentheses, is the percent identity and the ratio of the number of identities (i.e. the numerator) over the length of the amino acid sequence that was compared (i.e. the denominator) (Id.). The number outside of the parentheses is the score obtained in the Blast program (i.e. even a score of 1×10^{-9} is a sufficiently high score to identify the homologous gene) (Id.).

A similar search with the δ subunit of *E. coli* identified the holA gene of *Nisseria* and *Thiobacillus* as high matches, and holA of other enteric bacteria produced high scores as well (Supp. O'Donnell Decl. ¶23). Repetition of this procedure using *Neisseria* δ easily allows the identification of δ in *Aquifex aeolicus* (Id.). Use of *Aquifex aeolicus* δ identifies δ of *Enterococcus* (which identifies *Bacillus* δ, then *Streptococcus* δ, then *Synechocystis*, and the *Porphyromonas* δ) (Id.). Use of *Aquifex aeolicus* δ also identifies *Thermatoga* δ, which identifies Spirochaetes (*Borrelia*) δ subunit (Id.). Use of *Thiobacillus* δ identifies δ from *Helicobacter camylobacter* (Id.). There is a region at about 100 residues that is rather well conserved in δ across eubacteria and if this were used, the scores could be even higher yet (Id.). Figure 2 shows this "walking" procedure and shows the scores and percent identities obtained as a result of this procedure starting from the δ subunit of *E. coli* as well as alignments (Id.) (Appendix U attached to the Supp. O'Donnell Decl.). This figure is substantially the same as Figure 1 but within the parentheses, after the percentage identity, there is another ratio and another percentage based on homologies (Id.). Figure 2 does not show scores for individual Gram negative bacteria of the *Enterobacteria* class (called enterics) as they are highly related to *E. coli* and the scores are very high (Id.).

Therefore, those of ordinary skill in the art, using the sequence information in the present application, would have been able to (and, in fact, did) identify and isolate the δ and δ' subunits of polymerase III holoenzyme (and their encoding genes) from organisms other than *E. coli* (See Supp. O'Donnell Declaration ¶ 24).

Further, the sequence of the eubacterial homologues to δ', and indeed the other δ' homologues, are sufficiently homologous to the δ' subunit of *E. coli* to provide for identifying and obtaining the corresponding δ' (holA) gene from these organisms using the gene encoding the δ' subunit of *E. coli* in the following ways: (1) use of the *E. coli* holA gene, or fragments of the *E. coli* gene, as a probe in a Southern analysis of whole cell DNA

from another organisms to identify the corresponding δ' homologue; (2) use of holA, or its fragments, as a probe to screen cDNA plasmid libraries of other organisms; (3) use of the holA gene sequence to synthesize oligonucleotide primers for PCR to amplify the corresponding δ' homologue from total genomic DNA from other organisms; and (4) use of the holA gene sequence to identify the δ' homologue from a genome sequencing project of other organisms by sequence comparison to the *E. coli* holA gene (O'Donnell Declaration ¶ 14).

In addition, claims 5, 14, 54, and 55 have been limited to DNA molecules and protein subunits encoded by DNA molecules which comprise a nucleic acid sequence which hybridizes to a nucleotide sequence corresponding to SEQ. ID. No. 6 or SEQ. ID. No. 11 or SEQ. ID. No. 12 or SEQ. ID. No. 13 when hybridization is performed in 2 x SSC, 0.2% SDS at 42 °C. The use of such hybridization conditions restricts the claims to a limited set of variants. Thus, one of ordinary skill in the art could determine how many changes in nucleotide positions can occur and where such changes can occur as a result of the claimed hybridization conditions. Accordingly, the claimed genus's do not embrace widely variant species.

Further, as claims 5 and 59 are directed to an isolated DNA molecule encoding a subunit of polymerase III holoenzyme and claims 14, 19, 20, and 54 are directed to isolated protein subunits of a polymerase III holoenzyme, only particular proteins and particular DNA molecules are encompassed by the claims. In particular, only isolated DNA molecules encoding a subunit of polymerase III holoenzyme that hybridize to a particular nucleotide sequence under particular hybridization conditions and the isolated protein subunits of a polymerase III holoenzyme encoded by such DNA molecules are encompassed by the claims.

Moreover, claims 19 and 20 are directed to "[a]n isolated protein subunit of polymerase III holoenzyme, wherein the subunit has an amino acid sequence corresponding to amino acid residues 1-158 of SEQ. ID. No. 10" and "[a]n isolated protein subunit of polymerase III holoenzyme, wherein the subunit has an amino acid sequence corresponding to amino acid residues 107-158 of SEQ. ID. No. 10," respectively. Thus, these claims are limited to a particular disclosed sequence, fully described in the specification, as filed.

In view of the known structural and functional homology of the δ' and δ subunit proteins from various sources such as numerous other prokaryotes, the limitation of claims 19 and 20 to specific amino acid sequences, and the limitation of claims 5, 14, 54, and 59 to DNA molecules and protein subunits encoded by DNA molecules which comprise a

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nucleic acid sequence which hybridizes to a nucleotide sequence corresponding to SEQ. ID. No. 6 or SEQ. ID. No. 11 or SEQ. ID. No. 12 or SEQ. ID. No. 13 when hybridization is performed in 2 x SSC, 0.2% SDS at 42 °C, applicant has provided written description for the independent claims and any claims which depend therefrom. Accordingly, the rejection of claims 5, 7, 12, 13, 14, 16, 19, 20, 54, 59, 64, and 65 under 35 U.S.C. § 112, first paragraph, for lack of written description is improper and should be withdrawn.

The rejection of claims 5, 7, 12, 13, 14, 16, 19, 20, 54, 59, 64, and 65 under 35 U.S.C. § 112, first paragraph, for lack of enablement is respectfully traversed.

In particular, as described above, it is well known to one skilled in the art that proteins homologous to the δ and δ' subunits of the *E. coli* polymerase III holoenzyme are contained in organisms other than *E. coli*. Further, only isolated DNA molecules encoding a subunit of polymerase III holoenzyme that hybridize to a particular nucleotide sequence under particular hybridization conditions and the isolated protein subunits of a polymerase III holoenzyme encoded by such DNA molecules are encompassed by claims 5, 14, 54, and 59 and their dependent claims. Moreover, claims 19 and 20 are limited to specific amino acid sequences fully disclosed in the specification.

In addition, the present application fully discusses the isolation and sequencing of the δ' and δ subunits and their encoding genes for the polymerase III holoenzyme. In view of the disclosure of these experimental procedures, the known structural and functional homology of the δ' and δ subunits proteins from various sources such as numerous other prokaryotes, the limitation of claims 19 and 30 to specific amino acid sequence, and the limitation of claims 5, 14, 54, and 59 to DNA molecules and protein subunits encoded by DNA molecules which comprise a nucleic acid sequence which hybridizes to a nucleotide sequence corresponding to SEQ. ID. No. 6 or SEQ. ID. No. 11 or SEQ. ID. No. 12 or SEQ. ID. No. 13 when hybridization is performed in 2 x SSC, 0.2% SDS at 42 °C, it would not require an undue amount of experimentation for one skilled in the art to isolate and sequence the claimed δ' and δ proteins (and their encoding genes) from sources other than *E. coli*.

Accordingly, the rejection of claims 5, 7, 12, 13, 14, 16, 19, 20, 54, 59, 64, and 65 under 35 U.S.C. § 112, first paragraph, for lack of enablement is improper and should be withdrawn.

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In view of the all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Aug 8, 2001 — Date

Ruth R. Smith — Ruth R. Smith

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Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made herein to claims 17, 19, 55, and 60, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

17. (Amended) The isolated protein subunit according to claim 14, wherein the isolated protein subunit comprises an [corresponds to an] amino acid sequence of [corresponding to] SEQ. ID. No. 10.

19. (Twice-Amended) An isolated protein subunit of polymerase III holoenzyme, wherein the subunit has an amino acid sequence corresponding to amino acid [acids] residues 1-158 of SEQ. ID. No. 10.

55. (Amended) The isolated protein subunit of polymerase III holoenzyme according to claim 54, wherein the isolated protein subunit comprises [corresponds to] an amino acid sequence of [corresponding to] SEQ. ID. No. 9.

60. (Amended) The isolated DNA molecule according to claim 59, wherein the isolated DNA molecule comprises [corresponds to] a nucleotide sequence of [corresponding to] SEQ. ID. No. 6.

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